INTRODUCTION:

Nature has provided a complete storehouse of remedies to cure ailment of mankind. Medicinal plants have been used for centuries as remedies for disease because they contain component of therapeutic values. According to the WHO, 80% of the world population continues to rely mainly on traditional medicines for their health care (WHO, 1993). Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of bioactive ingredients and their ability to treat various diseases. (Cooper, 2004).

The aim of the study is to evaluate and compare the Phytochemical, antibacterial and immunomodulatory effects of *Spheranthus indicus* (SP), *Tinospora cordifolia* (TC) and *Kalanchoe pinnata* (KP) on urinary tract infection (UTI)

UTI is a bacterial infection of the Urethra, Ureters and Urinary bladder (cystitis) or even the Kidneys (Pyelonephritis). Presence of at least 10^5 colony forming units(CFU) per milliliter of freshly passed urine is regarded as evidence of likely UTI. Urinary tract infections (UTIs) are among the most commonly observed infections in clinical practice, and more than 25% of all women experience an UTI at least once during their lifetimes .The majority of UTIs are far from severe; i.e., they are not life-threatening and do not produce any irreversible damage. However, when the kidneys are involved in the infection, there is a risk of irreversible kidney tissue damage as well as an increased risk of bacteremia (Bergeron 1995*)*

Sphaeranthus indicus (Astraceae) commonly known as Gorakhmundi (Hindi) is an annual spreading herb which grow approximately 15-30 cm in height. The plant is distributed throughout the plains and wet-lands of India, Sri Lanka and Australia. Used traditionally for the treatment of jaundice, leprosy, fever, pectoralgia, cough, gastropathy, hernia, haemorrhoids, helminthiasis, dyspepsia, skin diseases and as a nerve tonic, the plant is known to possess varied medicinal properties and is reportedly used in Ayurvedic preparations for treating epileptic convulsions, mental illnesses and hemicranias (Ambavade *et al*., 2006; Jha *et al*., 2010). Its use in the Indian traditional folk medicine is also well documented. This plant is used in folk medicine as remedy for Urinary Tract Infections (Kritikar & Basu, 1935). Phytochemical analysis of *S. indicus* carried out revealed the presence of carbohydrates, proteins, amino acid**s,**

tannins, phenols, steroids, fats & oils and alkaloids.(Shirwaikar *et al*., 2006; Ambavade *et al*., 2006).

Tinospora cordifolia (Menispermaceae) known as Amrita or Guduchi is widely used by folk and Aurvedic system and Rasayanas to improve the immune system and body resistance against infections. The stem of the plant is greyish brown-black in colour and bitter in taste. The stems of *Tinospora cordifolia* are rather succulent with long filiform fleshy aerial roots from the branches. The stem is soft wooded, dry, cylindrical and 5mm to 25mm in diameter.(Kritikar & Basu, 1935). Traditionally, the plant has been in use as an antispasmodic, anti-inflammatory, jaundice, diabetes, seminal weakness, urinary tract infections, fever, general debility, skin diseases, expectorant, carminative, digestive, anti-stress and aphrodisiac. (Farooqi . et al., 2001).A variety of chemical constituents have been isolated from this plant and their structures have been established. The active ingredients include alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. (Javeed Ahmed et al., 2011).

*Kalanchoe pinnata (*Crassulaceae) is commonly known as patharkuchi. It is a erect perennial glabrous herb with woody stem and thick, succulent leaves having crenate margins, often with foliar buds and adventitious roots, grown in gardens all over the country. (Ghani 2003). The plant contains bersaldegenin-1, 3, 5-orthoacetate (Yan et al., 1992) and bufadienolide- bryophyllin-B(Yamagishi T. et al., 1989).Other chemical constituents from the plants bryophyllol, bryophyllone, bryophyllenone, bryophynol are also isolated (Siddiqui et al., 1989). The leaves and bark are bitter tonic, astringent to bowels, analgesics, carminative and useful in diarrhoea and vomiting. Antiulcer activities of the leaf were also reported (Pal et al; 1991). The plant has hepatoprotective activity and is also used to increase vascular integrity to treat hypertension and kidney stone sand to enhance the dropping of umbilical cord of a newly born baby (Ekpendu, 1998).The leaves of the plant are eaten to control diabetes(Lans,2006). They are diuretic, and applied to wounds, boils and bites of insects. (Okwu, 2006).

Materials and Methods

The plants used for the present study are

(1) *Sphaeranthus indicus* L.

(2) *Kalanchoe pinnata* (Lam.) Pers.

(3) *Tinospora cordifolia* (Willd.) Miers.

1) *Swaras bhawana:*.

Swaras Bhavna of 1,5 and 10 of *Sphaeranthus indicus* was given and phytochemical and biological changes occurring were checked . TLC method was adopted for phytochemical changes and antibacterial activity was check by agar well diffusion method (Arora, 2001).

2) Preliminary Phytochemical study .

Preliminary Qualitative phytochemical study of methanolic and aqueous extract of all the taxa was done, the following phytochemical test were performed. (Harbone 1984).

1.Carbohydrates
2.Proteins
3.Tannins
4.Alkaloids
5. Flavanoids
6. Oil content
7.Terpenoids
8. Glycosides
9. Steroids

3) Quantitative Phytochemical estimation:

Chemical investigation of *Sphaeranthus indicus, Kalanchoe pinnata*, and *Tinospora cordifolia* were carried out. The methods employed for estimating different parameters were as follows:

1. Tannins (Folin Denis method of Schandri, 1970)
2. Phenol (Folin Denis method of Swain & Hills, 1989)
3. Crude alkaloid content(Sairam & Khanna,1971).
4. Oil Content

4) Separation of Secondary metabolites by HPTLC:

Phytochemical analysis was done with the help of TLC and HPTLC method Wagner and Bladt(1996).

a)*Sphaeranthus indicus*

Essential oil : Strong blue with Spray reagents anisaldehyde-sulphuric acid in Solvent system Toluene: Ethylacetae(93:7)

Alkaloids: Blue, blue-green or violet in UV-365 nm Toluene: Ethlyacetate:

di ethylamine (70:20:10)

b)*Kalanchoe pinnata*

Flavanoid: Light Blue flouorescent zone seen in UV-365 nm Ethlyacetate: Formic acid :glacial acetic acid : water (100:11:11:26)

c)*Tinospora cordifolia*

Glycoside **:** Blue, brown, green and yellowish flouorescent zone seen in UV-365 nm the same zone appear brown or blue in day light.

Ethlyacetate:methanol : water(100:13.5:10)

5) Isolation of the Essential oil

a) Hydrodistillation Method

Plant powder of *Sphaeranthus indicus* was subjected to hydrodistillation for 4 h using a Clevenger- type apparatus (Mukherjee,2002).

b) Solvent extraction :

Plant powder of *Sphaeranthus indicus* was subjected to petroleum ether extraction for essential oil seperation. (Mukherjee,2002).

6) GS-MS analysis :

GS-MS analysis was done to identify the components of essential oil.

7) Antibacterial activity :

Chloroform, Methanol, Ethanol & Water were used as solvent for extract preparation for assessing antibacterial activity. The organism used for the study was *Escherichia coli, Klebsiella pneumoniae, Proteus mirabils, Aceto bector* and *Pseudomonas aeroginosa* which were isolated from the patients urine sample. Agar well diffusion method was used to determine the antibacterial activity of the extracts, Kanamycin well known antibiotic was used as the positive control. (Perez. et al.; (1990)

8) Bioautography

In this form of assay methanol extract of *Sphaeranthus indicus* was run on a TLC plate which was covered by nutrient broth medium seeded with the 100µl E-coli Bacteria to investigated the antibacterial activity of particular isolated compounds (Mukherjee,2002)

9) InVivo studies:

Outbred female wistar rats were used throughout the study and *Escherichia coli* used as pathogen which was isolated from the urine of patient suffering from urinary tract infection and identified by conventional method. Methanolic extract of three plant for three

different concentrations and aqueous extract of only *Sphaeranthus indicus L.* were administered orally for 15 days (Hanne. et al.,2000)

a) Bacteriological examination: After five days of plant extract administration assessment of bacterial inhibition in treated animals was carried out. Urine was collected by gentle compression of the bladder through the abdominal wall. A sample of urine was collected from the external urethral meatus by using a micropipette and 1 µl was inoculated on agar plate.

b) Assessment of Immunological Effects : After 15 days of treatment with herbs extract (SP, TC and KP) haematological parameters - Immunoglobulin G (IgG) and Immunoglobulin M (IgM) were evaluated for assessing the immunomodulatory effect of the plant extract.

c) Histopathalogical Study : The kidneys urinary bladder and liver were removed from rats body of the end of the treatment. The section of each organ were obsvered for histopathalogical study.

Results

Swaras *bhawana* of *Sphaeranthus indicus L.*

Bhawna-1 has shown increased antibacterial activity than the original plant powder. However, there was no further changes in the activity.

TLC analysis of the powder drug and *Bhawna* drug has shown presence of triterpenoids, essential oil, flavanoids and alkaloids.

Preliminary Phytochemical study

From the phytochemical investigations it was observed that proteins, alkaloids, tannins flavonoids, glycosides triterpenoid and sugars were present in all the three plants, while essential oil was present only in *Sphaeranthus indicus* while steroids were absent. But essential oil was absent in *Kalanchoe pinnata*. and *Tinospora cordifolia* steroid were present.

Quantitative estimation of Separation metabolites:

In quantitative estimation higher amount of crude alkaloids, phenols, tannins and oil content was *present in the Sphaeranthus indicus* then in *Kalanchoe pinnata* and *Tinospora cordifolia*.

Phytochemical Screening by TLC and HPTLC :

a)Sphaeranthus indicus .

Essential oil : Strong blue indicated the presence of essential oil in visible light.
Alkaloids : Blue, blue-green or violet in UV-365 nm indicated the presence of alkaloids.

b)Kalanchoe pinnata

Flavanoid : Light Blue flouorescent zone seen in UV-365 indicated the presence of flavanoids.

c)Tinospora cordifolia .

Glycoside : Blue, brown, green and yellowish flouorescent zone seen in UV-365 nm the same zone appear brown or blue in day light indicated the presence of glycoside.

Essential oil by Hydrodistillation Method :

The oil yield v/w on moisture free basis of aerial part was 0.06%(v/w)

Major compounds found by GC-MS analysis in the oil of *Sphaeranthus indicus are.*

*1)*Benzene,2-terta-butyle-1-4dimethoxy.
2) Edesm-4(14)en-11-ol.
3) n- Hexadecanoic acid.
4) n-Tetratetraacontane

Essential oil by Petroleum ether extraction

Major compounds found by GC-MS analysis in the oil of *Sphaeranthus indicus. are.*

*1)*Benzene,2-terta-butyle-1-4dimethoxy.
2) Edesm-4(14)en-11-ol.
3) 10 a-Ambros-11(13)-en-12-Oic acid 8 a- hydroxyl-4-oxo-c- lactone
4) 9,12-Octadecadienoic acid

Antibacterial activity :

a)Sphaeranthus indicus

It was found that the methanolic extract possessed high degree of antibacterial activity against *Escherichia coli* . Against *Klebsiella pneumonia* ethanolic extract of plant showed higher activity than any other extract. Against *Proteus mirabils,* Chloroformic extract show similar inhibition zone as that with Kanamycin. Against *Acetobector* and *Pseudomonas* methnolic and ethanolic extract shows similar activity, however it was higher than chloroform extract.

b) Kalanchoe pinnata

The methanolic & ethanolic extract of *Kalanchoe pinnata* showed significant antibacterial activity against *E.coli & Pseudomonas than other extract.* However it is less than *Sphaeranthus indicus*

c)Tinospora cordifolia .

The methanolic & ethanolic extract of *Tinospora cordifolia* showed significant antibacterial activity against *E. Coli, Acetobector, Klebsiella pneumonia, Pseudomonas* bacteria than other extract, However it is less than *Sphaeranthus indicus*

Bioautography

Zone of the inhibition has shown at three different Rf value. One of the compound was identified as alkaloids fraction of *Sphaeranthus indicus*

InVivo studies:

Bacteriological examination

Sphaeranthus indicus is potentially rich in antibiotically active compounds so, this plant gives effective result to decrease the bacterial count in urine sample, and TC and KP are not antibiotically effective plant but these both plant are immunomodulator. So, these plants are also decrease bacterial count through increasing immunity. Therefore After fifteen days of plants extract treatments effective result are found in all three plant

Assessment of Immunological Effects :

After 15 days of treatment of the infected rats with herbs extract (SP, TC and KP) levels of immunoglobulin G and M were estimated. In the disease control group immunity level has decrease than the normal control group. Immunoglobulin M (IgM) evel and Immunoglobulin G (IgG) level has increased in the group of animal treated with aqueous extract of *Sphaeranthus indicus.* The group treated with methanolic extract of SP, TC and KP has also shown dose response increase in immunoglobulin levels.

Histopathalogical studies:

Histopathalogical changes of Kidney, liver and Urinary tract were observed. In the disease control group the kidney and urinary bladder histology showed considerable damage. The group of animals treated with aqueous extract of *Sphaeranthus indicus* for fifteen days no

such pathological damage were observed. The group treated with methanolic extract of SP, TC and KP have however shown some abnormalities in the tissue. This observation is an indication of very effective kidney and urinary bladder protection against bacterial infection by *Sphaeranthus indicus* specially by aqueous extract.

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(Guide)

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